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Insulin-releasing and insulin-like activity of the traditional anti-diabetic plant Coriandrum sativum (coriander)

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Coriandrum sativum (coriander) has been documented as a traditional treatment of diabetes. In the present study, coriander incorporated into the diet (62.5 g/kg) and drinking water (2.5 g/l, prepared by 15 min decoction) reduced hyperglycaemia of streptozotocin-diabetic mice. An aqueous extract of coriander (1 mg/ml) increased 2-deoxyglucose transport (1.6-fold), glucose oxidation (1.4-fold) and incorporation of glucose into glycogen (1.7-fold) of isolated murine abdominal muscle comparable with 10^{-8} M-insulin. In acute 20 min tests, 0.25–10 mg/ml aqueous extract of coriander evoked a stepwise 1.3–5.7-fold stimulation of insulin secretion from a clonal B-cell line. This effect was abolished by 0.5 mM-diazoxide and prior exposure to extract did not alter subsequent stimulation of insulin secretion by 10 mM-L-alanine, thereby negating an effect due to detrimental cell damage. The effect of extract was potentiated by 16.7 mM-glucose and 10 mM-L-alanine but not by 1 mM-3-isobutyl-1-methylxanthine. Insulin secretion by hyperpolarized B-cells (16.7 mM-glucose, 25 mM-KCl) was further enhanced by the presence of extract. Activity of the extract was found to be heat stable, acetone soluble and unaltered by overnight exposure to acid (0.1 M-HCl) or dialysis to remove components with molecular mass <2000 Da. Activity was reduced by overnight exposure to alkali (0.1 M-NaOH). Sequential extraction with solvents revealed insulin-releasing activity in hexane and water fractions indicating a possible cumulative effect of more than one extract constituent. These results demonstrate the presence of antihyperglycaemic, insulin-releasing and insulin-like activity in Coriandrum sativum.

Coriander: Insulin: Diabetes mellitus

Before the discovery of insulin in the early 1920s and the later development of oral hypoglycaemic agents, the major form of treatment of diabetes mellitus involved starvation, dietary manipulation and the use of plant therapies (Bailey & Flatt, 1990). More than 400 plants worldwide have been documented as beneficial in the treatment of diabetes (Bailey & Day, 1989; Swanston-Flatt et al. 1991; Gray & Flatt, 1997a). However, most of these await proper scientific or medical evaluation, and the World Health Organization (1980) has recommended accordingly that assessment of traditional plant treatments for diabetes merits further investigation.

Coriandrum sativum (coriander) has been reported to have a number of possible medicinal attributes including antispasmodic, carminitive and stomachic properties (Lust, 1986). Additionally, coriander has been advocated as an anti-diabetic remedy (Farnsworth & Segelman, 1971; Lewis & Elvin-Lewis, 1977). More recent studies have confirmed the antihyperglycaemic effect of coriander in streptozotocin-diabetic mice (Swanston-Flatt et al. 1990), suggesting that further studies are warranted on the antihyperglycaemic actions of this plant. Anti-diabetic agents can exert beneficial effects in the diabetic environment by improving and/or mimicking insulin action and/or by enhancing insulin secretion (Gray & Flatt, 1997b). To understand better the mechanisms by which coriander ameliorates hyperglycaemia, the present study investigated in vitro actions of aqueous extracts of coriander on glucose metabolism by isolated murine abdominal muscle and on insulin secretion by a clonal B-cell line (BRIN-BD11).

Materials and methods

Plant material

Dried coriander seeds were obtained from a commercial source in Birmingham, West Midlands, UK. Seeds were homogenized to a fine powder and stored at room temperature (20 ± 2°C) in opaque screw-top jars. Powdered coriander was used for incorporation into test animal diet. An aqueous...
extract of coriander was prepared by a method of decoction as described previously (Gray & Flatt, 1998a). In brief, 1 g powdered material was placed in 40 ml cold (distilled) water, brought to the boil, then removed from the heat source and allowed to infuse for 15 min. This suspension was filtered (Whatman no. 1) and the volume readjusted to 40 ml with distilled water. For in vivo studies 10 ml portions of extract were stored at –20°C until use when they were diluted tenfold with tap water (2.5 g/l). For in vitro studies 1 ml portions of extract were brought to dryness under vacuum (Savant speedvac; Savant Instrumentation Incorp., Framingdale, NY, USA), stored at –20°C and reconstituted on the day of use with incubation buffer. Incubations within a single experiment using isolated muscle or BRIN-BD11 cells were always conducted using the same batch of extract. This allowed for any variation in potency of different batches of extract.

Animal studies

Heterozygous lean (ob/+ ) male mice derived from a colony maintained at Aston University, Birmingham, UK (Flatt & Bailey, 1981) were used at 21–24 weeks of age. Groups of two or three mice were housed in an air-conditioned room at 22 ± 2°C with a lighting schedule of 12 h light (08.00–20.00 hours) and 12 h dark. Animals had free access to a standard pellet diet (Mouse Breeding Diet, Pillsbury Ltd, Birmingham, W. Midlands, UK) and tap water. The overall nutrient composition of the diet was (g/kg): carbohydrate 362, protein 209, fat 44 and fibre 385 with a metabolizable energy content of 11.8 MJ/kg. The experimental procedure for in vivo studies was similar to that previously described (Gray & Flatt, 1997b). For animal diets, coriander was incorporated into powdered mouse diet and mixed thoroughly, then distilled water was added and the mixture was combined to a stiff paste. The diet was then pelleted and placed at 45°C until dry. Control diet was prepared by the same method to ensure there were no end differences in vitamin and mineral content as a result of the drying process. Coriander was incorporated into the diet (62.5 g/kg) and drinking water (2.5 g/l) of a group of seven mice 5 d before and after intraperitoneal administration of streptozotocin (STZ; Sigma Chemical Co., Poole, Dorset, UK) at 200 mg/kg body weight in 0.1 M sodium citrate buffer (pH4.5). Daily measures of body weight, food intake and fluid intake were made. Non-fasting blood samples obtained from the cut tail-tip of conscious mice were collected at the same time (09.00–10.00 hours) for plasma glucose analysis (Stevens, 1971). Groups of six normal mice and six STZ-treated mice with free access to unsupplemented diet and drinking water were used as controls.

Glucose transport and glucose metabolism in vitro

Recently-weaned non-fasting male mice (3–5 weeks) were killed by cervical dislocation and pieces of abdominal muscle (approximately 10–20 mg) were prepared. In order to replicate by mouse, pieces of muscle from each mouse were designated to each of the treatment groups. Glucose uptake was determined as described previously (Gray & Flatt, 1998b). In brief, muscle pieces were incubated at 30°C for 30 min in Krebs-Ringer bicarbonate buffer (KRB) supplemented with 2 mM-sodium pyruvate, 3.7 kBq/ml 2-deoxy-[-1-3H]glucose, 0.37 kBq/ml L-[-1-14C]glucose in the presence and absence of 10–8 M-human insulin and 1 mg/ml extract of coriander (2 × 2 factorial design). Oxidative glucose metabolism to CO2 and incorporation of glucose into glycogen were determined as described previously (Gray & Flatt, 1998b). In brief, muscles were incubated at 37°C for 60 min in KRB supplemented with 10 mM-glucose, 18.50 kBq/ml d-[U-14C]glucose in the presence and absence of 10–8 M-human insulin and 1 mg/ml extract of coriander (2 × 2 factorial design). Following incubation, CO2 was captured onto a NaOH-saturated filter paper and muscles were removed for glycogen analysis (Gray & Flatt, 1998b).

Insulin secretion in vitro

BRIN-BD11 cells, produced by electrofusion of immortal RINm5F cell with New England Deaconess Hospital rat pancreatic B-cell, were used to evaluate insulin secretion (McClenaghan et al. 1996; Gray & Flatt, 1997b). In brief, wells containing 0.2 × 106 insulin-secreting cells were washed with KRB containing 1.1 mM-glucose and preincubated for 40 min at 37°C (Gray & Flatt, 1997b). Unless otherwise stated, cells were then incubated for 20 min with KRB at 1.1 mM-glucose in the absence and presence of plant extract, diazoxide (an established opener of K+ -ATP channels) and other test agents. Following incubation, portions were removed from each well and stored at –20°C for insulin assay (Flatt & Bailey, 1981). Modified neutral red assay (Hunt et al. 1987) confirmed that 0.25–10 mg/ml coriander extract did not influence the viability of BRIN-BD11 cells during the test period.

To assess the importance of heat during extract preparation, aqueous extracts of coriander were prepared by the normal method of decoction (normal extract) or by cold infusion (cold extract; plant material placed in cold water, allowed to stand for 15 min, then filtered as before). Modified aqueous extract was freshly reconstituted in KRB and the effect on insulin secretion evaluated at a concentration equivalent to 1 mg/ml compared with normal extract (produced by 15 min decoction, as described previously). To further evaluate the nature of the insulin-releasing component(s), the normal aqueous extract of coriander prepared by decoction was subjected to heat, overnight dialysis, acid–alkali or acetone treatment. Heat: aqueous extract was boiled for 1 h immediately after preparation. Dialysis: aqueous extract was dialysed overnight (Spectra/Por molecular mass cut-off 2000 Da; Spectrum, Los Angeles, CA, USA) against deionized water (Milli-Q, Millipore Corp., Milford, MA, USA) at 4°C. Acid–alkali treatment: portions of aqueous extract were added to 5 M-HCl or 5 M-NaOH to produce 0.1 M-HCl or 0.1 M-NaOH, allowed to stand at room temperature overnight, then neutralized. Acetone treatment: 1 ml aqueous extract (1 mg/ml) was added to 10 ml ice-cold acetone, allowed to stand for 30 min (on ice) and centrifuged (1000 rev./min; 5 min) to obtain acetone-soluble and acetone-insoluble fractions. Portions of untreated extract and modified aqueous extracts were dried under vacuum. All modified
aqueous extracts were freshly reconstituted in KRB and effects on insulin secretion at a concentration equivalent to 1 mg/ml were compared with that of untreated extract.

In another series of experiments ground coriander seeds were subjected to sequential extraction by increasingly polar solvents. Plant material (0.25 g) was placed in 5 ml hexane, agitated for 15 min and centrifuged (1200 rev./min: 5 min). The precipitate was dried under vacuum, extracted with a further 5 ml hexane and centrifuged as before. The extraction supernatant fractions were pooled, filtered (Whatman no. 1) and the volume adjusted to 10 ml hexane. All extract fractions were brought to dryness under vacuum and freshly reconstituted in KRB for investigation of effects on insulin secretion at a concentration equivalent to 1 mg/ml.

Throughout the study levels of food intake by treatment groups did not differ.

Glucose transport and glucose metabolism in vitro
Aqueous extract of coriander (1 mg/ml) increased glucose uptake (1.6-fold), glucose oxidation (1.4-fold), and glycogenesis (1.7-fold) during incubations without insulin but did not significantly alter the stimulatory effect of 10^{-8} M insulin (Table 2).

Insulin secretion in vitro
Aqueous extract of coriander (0.25–10 mg/ml) had a dose-dependent 1.3–5.7-fold stimulatory effect on insulin secretion by BRIN-BD11 cells at 1.1 mM-glucose (Fig. 1). It was confirmed that these concentrations of extract did not influence the viability of BRIN-BD11 cells during the test period as evaluated by modified neutral red assay (Hunt et al).

Results
Studies in vivo
Compared with normal mice, STZ-treated mice showed significant (P < 0.05) weight loss, polydipsia and hyperglycaemia (Table 1). Administration of coriander in the diet and drinking water significantly decreased the hyperglycaemia by study day 20 (Table 1). Polydipsia increased in coriander supplemented v. unsupplemented STZ-treated mice (Table 1) and may indicate the presence of diuretic components in coriander seed. Plasma glucose concentrations of STZ-treated mice receiving coriander were comparable to those of normal mice at day 20 (Table 1). Throughout the study levels of food intake by treatment groups did not differ.

**Table 1.** Effects of coriander, administered in the diet (62.5 g/kg) and drinking water (2.5 g/l) on body weight, fluid intake, food intake and plasma glucose concentrations of streptozotocin (STZ)-treated mice†‡

<table>
<thead>
<tr>
<th></th>
<th>Normal mice</th>
<th>STZ mice</th>
<th>STZ mice + coriander</th>
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<tbody>
<tr>
<td></td>
<td>Mean</td>
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<td>Mean</td>
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<td>47.2</td>
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<td>37.9**</td>
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<td>Study day 20</td>
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<td>37.7**</td>
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<td>4.3</td>
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<td>Study day 20</td>
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<td>22.3**</td>
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Mean values were significantly different from those for normal mice: *P < 0.05, **P < 0.01, ***P < 0.001.

Mean values were significantly different from those for STZ-treated mice receiving normal diet: †P < 0.05, ††P < 0.01.

‡ For details of diets and procedures, see pp. 203–205. STZ was administered 4d after the introduction of coriander (day 0).
The presence of 0.5 mM-diazoxide inhibited the stimulatory effect of the extract (Fig. 2(a)), indicating that the enhancement of insulin release was not a mere consequence of cellular damage. Consistent with this view, prior exposure of BRIN-BD11 cells to extract for 20 min did not alter the subsequent insulin secretory response to 10 mM-L-alanine (Fig. 2(b)). The insulin-releasing effect of 1 mg/ml extract was markedly potentiated by the presence of high (16.7 mM) glucose (Fig. 3(a)) and by the presence of 10 mM-L-alanine (Fig. 3(b)). However, the action of extract (1 mg/ml) was not potentiated by 1 mM-3-isobutyl-1-methylxanthine, which increases cyclic AMP in insulin-secreting cells (Sharp, 1979) (Fig. 3(c)). In contrast, aqueous extract of coriander further enhanced the insulin release by depolarized BRIN-BD11 cells (16.7 mM-glucose, 25 mM-KCl) (Fig. 3(d)).

Temperature of extraction did not alter the effect of the extract in enhancing insulin secretion (results not shown). Prolonged exposure to heat, overnight dialysis or exposure to acid did not significantly alter the insulin-releasing effect of the plant extract (Table 3). Prolonged exposure to an alkaline environment reduced the insulin-enhancing effect (Table 3). The insulin-releasing activity was completely retained in the acetone-soluble fraction (Table 3). Both the hexane and water fractions of coriander (produced by sequential extraction) exerted an insulin-enhancing effect comparable with normal aqueous extract of coriander (Fig. 4). Although less potent than the normal aqueous extract, the ethyl acetate and methanol fractions of coriander (produced by sequential extraction) exerted insulin-releasing effects (Fig. 4).

### Discussion

Early experiments involving administration of coriander fruit as a decoction did not reveal effects on fasting blood...
sugar levels of normal and alloxan diabetic rats, but demonstrated alleviation of adrenaline-induced hyperglycaemia (Sharaf et al. 1963). Subsequent studies involving longer-term administration of coriander seed in the diet showed that the plant purported as a traditional treatment for diabetes indeed decreased the hyperglycaemia of STZ-diabetic mice (Swanston-Flatt et al. 1990). The present study not only confirms this antihyperglycaemic action but indicates the presence of natural products in coriander which exhibit insulin-releasing and insulin-like actions. The possibility also exists that dietary administration of coriander affords protection against STZ-induced pancreatic B-cell destruction, but this requires further evaluation.

The possibility that part of the glucose lowering action of coriander may be due to an effect on glucose handling by skeletal muscle was evaluated in vitro using an insulin-sensitive mouse skeletal muscle preparation (Gray & Flatt, 1998b). These experiments revealed that an aqueous extract of coriander enhanced glucose transport, glucose oxidation and glycogenesis to an extent comparable with 10⁻⁸ M-insulin. The combined actions of coriander and insulin on muscle were not additive, suggesting that the active constituent(s) of coriander extract acts through a pathway similar in part to that utilized by insulin. Interestingly, the action of coriander differs significantly from that of the established antihyperglycaemic drug, metformin, which exerts effects on glucose transport via insulin-mediated peripheral glucose uptake (Bailey & Puah, 1986; Prager et al. 1986).

Experiments using clonal pancreatic B-cells showed that coriander extract stimulated insulin secretion in a dose-dependent fashion over the concentration range 0.25–10 mg/ml. The maximal fivefold insulin response can be compared with an approximate doubling of insulin release in this cell line induced by micromolar concentrations of

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**Fig. 3.** Insulin secretion by BRIN-BD11 cells incubated in the presence or absence of aqueous coriander extract (1 mg/ml) with various other substances. (a) ○, 1.1 mm-glucose (control) and □, 16.7 mm-glucose; (b) ○, 1.1 mm-glucose (control) and □, 10 mm-L-alanine; (c) ○, 1.1 mm-glucose (control) and □, 1 mm-3-isobutyl-1-methylxanthine (IBMX); (d) ○, 16.7 mm-glucose (control) and □, 25 mm-KCl. Values are means for groups of six observations with their standard errors indicated by vertical bars. Two-way ANOVA revealed: (a) extract effect (P < 0.001), glucose effect (P < 0.001) and extract × glucose interaction (P < 0.004); (b) extract effect (P < 0.001), L-alanine effect (P < 0.001) and extract × L-alanine interaction (P = 0.021); (c) extract effect (P < 0.001) and IBMX effect (P < 0.001); (d) extract effect (P < 0.001), KCl effect (P < 0.001) and extract × KCl interaction (P = 0.02).
sulfonylureas, tobutamide and glibenclamide (McClenaghan et al. 1998; McClenaghan & Flatt, 1998). The stimulatory action of coriander extract was also glucose-dependent and potentiated by the established amino acid stimulator L-alanine. However, insulin release was not enhanced by the phosphodiesterase inhibitor 3-isobutyl-1-methylxanthine which increases cyclic AMP and enhances hormone-mediated insulin release (Sharp, 1979). Importantly, prior exposure to 0.5 mg/ml extract did not diminish the subsequent insulin secretory response to L-alanine, thereby negating a possible deleterious effect of extract on cell viability in the insulin secretion experiments. The non-toxic nature of coriander extract was also confirmed by toxicity testing using the modified neutral red assay.

Studies performed to evaluate further the possible mechanisms underlying the insulin-releasing action of coriander extract confirmed an action similar to that of sulfonylureas. These agents act by binding to sulfonylurea receptors, resulting in closure of plasma membrane K⁺-ATP channels, membrane depolarization, opening of voltage-dependent Ca channels and elevation of intracellular Ca²⁺ (Rorsman, 1997). Diazoxide, by holding open K⁺-ATP channels, inhibits the stimulatory action of sulfonylureas (Dunne et al. 1994). In the present study, diazoxide also inhibited the insulin-releasing action of coriander. Like sulfonylureas (Flatt et al. 1994; Eliasson et al. 1996) coriander also stimulated insulin release from chemically depolarized cells incubated with 25 mM-KCl. This suggests that constituents of coriander extract have K⁺-ATP channel and distal actions similar to sulfonylureas. However, the greater potency of coriander extract compared with maximally effective concentrations of sulfonylureas suggests possible additional actions on B-cell nutrient metabolism or second-messenger pathways.

Preliminary investigations into the nature of the insulin-releasing agent(s) in coriander indicate heat stability, acetone solubility and resistance to acidic or alkaline environments. Extract activity was not due to smaller molecules or ions as suggested by retention of potency following dialysis to remove components of low molecular mass (< 2000 Da). Studies using sequential solvent extractions of coriander seeds point towards cumulative insulin-releasing effects of more than one active constituent of coriander. The involvement of these or other extract components in mediating the effects of coriander on glucose uptake and metabolism clearly merit further investigation.

In conclusion, the present study has shown that the antihyperglycaemic action of coriander is associated with stimulation of insulin secretion and enhancement of glucose uptake and metabolism by muscle, reflecting the effects of more than one active constituent. Coriandrum sativum therefore represents a possible antihyperglycaemic dietary adjunct and potential source of a new orally active agent(s) for diabetes therapy.

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